

FATTY ACID COMPOSITION OF FAT DEPOTS IN WINTERING CANADA GEESE

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ABSTRACT.—I determined the fatty acid composition of subcutaneous, abdominal, visceral, and leg saddle depots in adult female Canada Geese (*Branta canadensis*) wintering in north-central Missouri during October 1984–March 1985. Mean levels of C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, and C18:3 generally were highest in the subcutaneous and abdominal depots. The ratio of saturated to unsaturated fats was highest in the leg saddle depot and lowest in the abdominal depot. I also assessed the differences among sexes, seasons, and years in fatty acid composition of abdominal fat depots in adult geese collected during October–March, 1985–1987. Adult females had consistently higher levels of C14:0 in abdominal depots than males. Fatty acid composition of the abdominal depot differed among years but not by season. In the abdominal depot, C14:0, C16:0, C16:1, and C18:1 were higher in 1986–1987 compared with the previous two years, whereas C18:3 was highest in 1984–1985. Differences among years reflected changes in winter diet. Fatty acids of wintering geese were similar to those previously found in breeding Canada Geese. *Received 2 Oct. 1992, accepted 22 Dec. 1992.*

Waterfowl use stored lipids to meet nutritional requirements during the migratory (Berthold 1975), breeding (Afton and Ankney 1991), and wintering phases of the annual cycle. In winter, lipid reserves provide insulation and energy for thermoregulation and may also serve as nutrient reserves during periods of severe weather or food shortages.

Lipids are stored primarily as triglycerides (Blem 1990). Characteristics vary with chain length (number of carbon atoms) and saturation (number of double bonds). Longer-chain fatty acids yield more energy during β -oxidation than short-chain fatty acids. Unsaturated fatty acids have a lower melting point and thus are more readily mobilized than saturated fatty acids (Johnston 1973). Fatty acid composition of adipose tissue in birds generally varies among seasons (West and Meng 1968, Johnson and West 1973, Yom-Tov and Tietz 1978, Middendorf 1981), but most seasonal differences have been attributed to fatty acid composition of food consumed (Blem 1990).

Lipid reserves are largely deposited in distinct depots within the body (e.g., subcutaneous, abdominal, or visceral depots). Investigators of fatty acid composition of birds have often reported the fatty acid composition of carcass fat, plasma lipids, or one or two depots but have infrequently

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evaluated differences among depots. Several studies comparing fatty acid composition among depot fats showed little difference (Johnson and West 1973, Thomas and George 1975, Yom-Tov and Tietz 1978), but Heitmeier and Fredrickson (1990) found differences in several common fatty acids.

The objectives of my study were to (1) compare fatty acid composition in the abdominal, visceral, leg saddle, and subcutaneous fat depots of adult female Canada Geese (*Branta canadensis*); (2) evaluate differences in fatty acid composition of abdominal depots among sexes, seasons, and years of adult Canada Geese wintering in Missouri; and (3) compare fatty acid composition of abdominal depots of geese in pairs and family groups. In the first objective, I hypothesized that the most labile fat depots contained a higher proportion of unsaturated fatty acids. In the second and third objectives, I hypothesized that fatty acid composition did not differ between sexes or within pairs and family groups, because geese feed together as family groups, but differed among seasons and years because of varying diets and habitat use.

STUDY AREA AND METHODS

The study was conducted at Swan Lake National Wildlife Refuge (NWR) in north-central Missouri (study area is described in Austin [1988]). Adult Canada Geese were collected from October through March, 1984–1987 by shotgun, rifle, and rocket net. Age and sex were determined from cloacal and plumage characteristics (Hanson 1962). Geese were frozen immediately and later were thawed for dissection. Fat samples were excised from each goose, weighed, and frozen in glass vials at -40°C until laboratory analysis. Analyses of fatty acids were performed by the Missouri Agriculture Experiment Station Chemistry Laboratory, Univ. Missouri–Columbia, using the methods of Gehrhardt and Gehrke (1977) with the following modifications. Each fat sample was weighed on a cotton square and placed in a glass thimble for a Goldfish extractor. The thimble samples were lyophilized for 15 h and extracted with diethyl ether for 4 h. The methyl ester derivative of each fatty acid was used in analyses. The extract was brought to 100 ml with diethyl ether at room temperature. A 1-ml aliquot of each sample was analyzed with 10% or more reruns to check precision and extreme values; for duplicate samples, averaged results were used in statistical analyses. A capillary gas-liquid chromatograph apparatus interfaced with a computer was used to identify fatty acids. Margaric acid was employed as the internal standard for quantifying fatty acids. The chromatographic column was a Supelcowax 10 fused silica capillary column, 30 m \times 0.25 mm ID; oven temperature was programmed from 100 to 200°C at 10°C/min. Results are expressed as mg/g.

To compare fatty acid composition among depots, samples of four fat depots were excised from each of 12 adult female geese collected during October 1984–March 1985. The depot sites were (1) abdominal, a fat pad covering the viscera beneath abdominal muscles; (2) visceral, fat adhering to intestinal mesentery; (3) leg saddle, a distinct depot overlaying the knee of the right leg; and (4) subcutaneous, a depot from the underside of the skin in the intraclavicle area.

I used a multivariate analysis of variance (MANOVA) in a randomized complete block design to test the hypothesis that no differences existed in mean fatty acid composition

among the four depots. Birds served as blocks and depots as treatments. If the overall MANOVA was significant, I examined each of the fatty acids separately by univariate analysis of variance (ANOVA). If the F -test for depot was significant, I used Fisher's protected LSD value to determine which depot(s) differed (Milliken and Johnson 1984). I pooled fatty acids into saturated and unsaturated groups and also tested differences in saturation (ratio of saturated to unsaturated fatty acids) among depots with univariate ANOVAs. If the ANOVA was significant, I used Fisher's protected LSD value to determine which depot(s) differed (Milliken and Johnson 1984). All statistical analyses were conducted with SAS system programs (SAS Institute, Inc. 1990) with the significance level set at $P = 0.05$.

To compare fatty acid composition among sexes, years, and seasons, I selected 36 adult male and 42 adult female Canada Geese from 163 collected during October–March, 1985–1987. These individuals, which had been marked with neck collars, were selected because I had repeated sightings of them during the year they were collected. I excised and analyzed only the abdominal fat depot because it is the largest and most readily identifiable and accessible depot.

I tested the hypothesis that sex, year, and season had no significant effect on mean fatty acid composition. Because seven of the season-sex-year groups had no data and others had small sample sizes, I first evaluated the influence of season by Julian date (hereafter referred to as date) rather than by specific seasons. I assumed a completely randomized design and used a multivariate analysis of covariance approach with individual birds as the experimental units and date as a covariate. I first tested whether date was dependent on group (two sexes by three years), which would identify covariate interaction with group (e.g., all males were collected early in the year). Secondly, I ran a MANOVA to test dependency of fatty acids on date within the six groups. If the overall MANOVA test was significant, I examined each of the fatty acids separately using univariate analysis of variance (ANOVA). If the F -test for a depot was significant, I used Fisher's protected LSD value to determine which sexes or year(s) differed (Milliken and Johnson 1984).

I pooled fatty acids into saturated and unsaturated groups for each sample and tested for differences between sexes and among years in saturation (ratio of saturated to unsaturated fatty acids) using univariate ANOVAs. If the F -test was significant, I used Fisher's protected LSD value to determine which sexes or year(s) differed (Milliken and Johnson 1984).

Among the geese collected were five adult pairs and two geese each from three family groups. The pair or family status was known based on observations of neck bands during that year. Because pairs and families feed together throughout the nonbreeding season, I expected that fatty acid composition of fat depots would be similar within pairs and families and strongly correlated between the two birds in each group. I used Pearson's product moment correlation to examine relations between birds for each of seven fatty acids.

RESULTS

Myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids were detected in all samples (Table 1). Lauric acid (C12:0) was detected in trace amounts in 12 of 92 abdominal samples ($\bar{x} = 0.72$ mg/g, $N = 90$) and in two of 12 visceral samples ($\bar{x} = 0.76$ mg/g). Arachidonic acid (C20:4) was detected in only two (<2%, $N = 126$) of all depot samples (3.7 and 12.3 mg/g).

Depots differed in mean levels of fatty acids (Wilks lambda = 0.100, $F = 4.58$, $df = 21, 78$, $P = 0.0001$) (Table 1). Only C14:0 did not differ

TABLE 1

FATTY ACIDS (MG/G) IN FOUR FAT DEPOTS OF ADULT FEMALE CANADA GEESE (N = 12) COLLECTED AT SWAN LAKE NATIONAL WILDLIFE REFUGE, NORTH CENTRAL MISSOURI, DURING OCTOBER–MARCH, 1983–1984

Fatty acid	P*	Subcutaneous		Abdominal		Visceral		Leg saddle	
		\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
C14:0	0.056	2.7 ^a (0.4)	0.1	2.9 ^a (0.4)	0.1	2.6 ^a (0.4)	0.2	2.4 ^a (0.4)	0.2
C16:0	0.036	145.3 ^b (20.3)	8.5	140.3 ^{ab} (20.0)	8.7	129.4 ^a (20.0)	8.0	126.3 ^a (20.5)	8.5
C16:1	0.001	20.1 ^b (2.8)	2.3	23.6 ^c (3.4)	2.7	19.0 ^b (3.0)	1.9	15.9 ^a (2.6)	2.0
C18:0	0.010	57.2 ^b (8.0)	2.7	49.1 ^a (7.0)	2.3	48.6 ^a (7.8)	3.1	52.4 ^{ab} (8.5)	2.6
C18:1	0.003	344.8 ^b (48.1)	14.3	335.7 ^b (47.8)	15.1	302.2 ^a (47.1)	17.8	295.5 ^a (48.0)	15.4
C18:2	0.003	128.5 ^b (17.9)	19.1	131.6 ^b (18.7)	20.4	121.4 ^b (18.9)	19.1	107.9 ^a (17.5)	16.0
C18:3	0.005	18.4 ^b (2.6)	3.1	19.6 ^b (2.9)	2.9	18.3 ^b (2.9)	3.3	15.8 ^a (2.6)	2.8

* P values from univariate ANOVA to test H_0 : no difference in mean fatty acid level among depots.

^{a,b,c} Values within each fatty acid followed by different superscript letters are different ($P < 0.05$) based on Fisher's LSD test. Percentage of total detected fatty acids are in parentheses.

among depots, although the result was marginal ($F = 2.79$, $df = 3, 33$, $P = 0.056$). Mean levels of most fatty acids were highest in subcutaneous and abdominal depots and lowest in visceral or leg depots. Visceral and leg saddle depots contained a larger proportion of nonextractable residues, such as additional connective tissues, mesentery, and blood vessels. Mean total detected fatty acids were 716.9 mg/g in the subcutaneous depot, 702.7 mg/g in the abdominal depot, 641.6 mg/g in the visceral depot, and 616.3 mg/g in the leg saddle depot.

The ratio of saturated to unsaturated fatty acids also differed among depots ($F = 17.52$, $df = 3, 33$, $P = 0.0001$). Leg saddle depots contained the highest mean ratio of saturated to unsaturated fatty acids (0.421), followed by the subcutaneous (0.407), visceral (0.402), and abdominal depots (0.381).

Fatty acid composition of sex-year groups showed no significant difference ($F = 1.1$, $df = 5, 73$, $P = 0.369$) by date. Because the MANOVA to test dependency of fatty acids on dates was marginally nonsignificant (Wilks lambda = 0.408, $F = 1.43$, $df = 42, 285$, $P = 0.051$), I tested the relationship between date and individual fatty acids. I detected a weak relation between date and individual fatty acids, which were inconsistent

from year to year. Therefore, I concluded that none of the fatty acids depended significantly on date and proceeded to run a MANOVA, ignoring date, to test for the effects of year and sex.

Fatty acid composition of the abdominal depot was affected by year (Wilks lambda = 0.412, $F = 5.26$, $df = 14, 132$, $P = 0.0001$) and sex (Wilks lambda = 0.743, $F = 3.26$, $df = 7, 66$, $P = 0.005$). I found no interaction between year and sex (Wilks lambda = 0.861, $F = 0.73$; $df = 14, 132$; $P = 0.737$). Only C14:0 differed among sexes ($P = 0.023$) when each fatty acid was examined independently. Females consistently had higher levels than males (3-year least squares means [LSMEANS] \pm SE of 3.32 ± 0.11 vs 2.96 ± 0.11 , respectively).

Levels of five of seven fatty acids (C14:0, C16:0, C16:1, C18:1, and C18:3) differed among years (Table 2). C14:0, C16:0, C16:1, and C18:1 were significantly higher in 1986–1987 than in the previous two years. This pattern was reversed for C18:3, which was markedly higher in 1984–1985 than in the later two years.

The ratio of saturated to unsaturated fats was not dependent on date ($F = 1.27$, $df = 6, 66$, $P = 0.286$), so date was not included in the model for MANOVA tests. Saturation differed among years ($F = 3.84$, $df = 2, 72$, $P = 0.026$) but did not differ between males and females ($F = 1.13$, $df = 1, 72$, $P = 0.291$). Pair-wise comparisons among years revealed that the only year-to-year differences occurred between 1984–1985 and 1985–1986. I found no interaction between sex and year (Wilks lambda = 0.975; $F = 0.45$; $df = 4, 142$; $P = 0.772$). Saturation was lowest in 1984–1985 (LSMEANS = 0.401) but similar between 1985–1986 (0.442) and 1986–1987 (0.425).

Birds in pairs and families had similar fatty acid composition. No consistent differences in fatty acid composition were apparent by sex within pairs or families except for C18:1, for which adult females tended to have slightly lower levels than their mates. Four fatty acids (C16:0, C18:1, C18:2, and C18:3) showed high correlations as expected between the two birds within the pair or family ($r > 0.50$, $P \leq 0.10$ [P values indicate whether r differed significantly from 0]). C14:0, C16:1, and C18:0 showed weaker correlations ($r = 0.37$, $P = 0.344$; $r = 0.51$, $P = 0.19$; and $r = 0.33$, $P = 0.33$, respectively). One pair, which included a radio-equipped female, had been separated by 12 km for two weeks in late winter before reuniting. This pair differed only slightly in fatty acid composition; C18:1 was higher in the male (374 vs 281 mg/g).

DISCUSSION

The results of my study, if expressed as a percentage of total fatty acids, are similar to those of Thomas and George (1975). Using this expression

TABLE 2

FATTY ACID COMPOSITION (MG/G). EXPRESSED AS LSMEANS, OF ABDOMINAL FAT DEPOTS IN ADULT CANADA GESE COLLECTED AT SWAN LAKE NATIONAL WILDLIFE REFUGE, NORTH CENTRAL MISSOURI, DURING OCTOBER–MARCH, 1985–1987

Fatty acid	P*	1984–1985 (N = 32)		1985–1986 (N = 26)		1986–1987 (N = 20)	
		\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
C14:0	0.006	2.9 (0.4)	0.1 ^a	3.0 (0.4)	0.1 ^a	3.5 (0.4)	0.2 ^b
C16:0	0.005	144.4 (20.3)	6.1 ^a	154.1 (21.5)	6.4 ^a	177.1 (21.9)	7.6 ^b
C16:1	<0.001	19.9 (2.8)	1.3 ^a	22.0 (3.1)	1.4 ^a	29.7 (3.7)	1.6 ^b
C18:0	0.252	56. (8.0)	2.3	62.2 (8.7)	2.4	60.9 (7.5)	2.9
C18:1	0.001	349.3 (49.1)	12.5 ^a	367.5 (51.3)	13.2 ^a	425.4 (52.5)	15.8 ^b
C18:2	0.286	117.2 (16.5)	7.0	101.0 (14.1)	7.4	107.7 (13.3)	8.9
C18:3	<0.001	20.6 (2.9)	1.6 ^b	6.2 (0.9)	1.7 ^a	5.9 (0.7)	2.1 ^a

* Significance levels (P) from univariate ANOVA to test H₀: no difference in mean fatty acid level among years.

^{a,b} Values for each fatty acid followed by different superscript letters are different (P < 0.05) based on Fisher's LSD test. Percentage of total detected fatty acids by mass are in parentheses.

of results, the most important differences in fatty acid composition between the two studies occur in postreproductive males. Males in the earlier study had a smaller proportion of C16:0 (16.9%) and C18:1 (34.1%) and larger proportions of C18:2 (30.4%) and C18:3 (12.2%) triglycerides, which are probably related to differences in dietary fatty acids (Thomas and George 1975:164).

Fatty acid composition of carcass or depot lipids is largely related to diet (e.g., Yom-Tov and Tietz 1978, Middendorf 1981, Heitmeyer and Fredrickson 1990). In this study, fatty acid composition of depot fat of migrant and wintering Canada Geese reflected the fatty acid composition of common dietary items, both native and agricultural. Corn, soybeans, and milo, the most commonly consumed agricultural grains, are high in C18:1 and C18:2 and relatively high in C16:0 (National Research Council 1984). Seeds of smartweed (*Polygonum*) and spikerush (*Echinochloa*) and tubers of yellow nutsedge (*Cyperus esculentus*), commonly consumed native moist-soil foods (Eggeman et al. 1989), are high in C14:0, C16:1, and C18:3 relative to corn and are similar to corn in their high levels of C18:1 and C18:2 (Heitmeyer and Fredrickson 1990). Green forage consumed

by geese, primarily young leaves of winter wheat, clover, and pasture grasses, provide C16:0, C18:0, and C18:3 fatty acids (Mead and Fulco 1976, Tinoco 1982).

Contrary to my results, Thomas and George (1975) found no difference in fatty acid composition of visceral and claviculo-coracoid depots for the same populations of premigrant (geese at Swan Lake NWR in early March), postmigrant (on arrival on the breeding grounds), and postreproductive geese (following nesting) that I studied. However, their results were based on small sample sizes, and, like other comparisons of fatty acid composition among depots (Johnson and West 1973, Yom-Tov and Tietz 1978, Middendorf 1981, Heitmeyer and Fredrickson 1990), were expressed as a percentage of total detected fatty acids rather than as mg/g of fat tissue (this study). When the mean values in Table 1 are expressed as percent of detected fatty acids, little difference in saturation is found among depots (0.305–0.312).

Although most highly saturated, the leg saddle was the most labile of fat depots in Canada Geese, as measured by the slope of the relationship between depot mass and total carcass lipid content (g) (Austin 1988). This is contrary to the expectation that the more labile depot would have the lowest level of saturation. Heitmeyer and Fredrickson (1990) found that the most labile depot of Mallards (*Anas platyrhynchos*) (abdominal) contained more C18:2 and C18:3 and had a low level of saturation, similar to that of the mallard leg saddle. In the Canada Geese I studied, the abdominal depot was the least saturated with high levels of C16:0 and C18:0. However, this depot also contained relatively high levels of C18:2 and C18:3 and seemed to be intermediate in terms of lability. Saturation of the subcutaneous depot was intermediate compared to the high saturation level in Mallards. This depot seems to be conservatively utilized in waterfowl (Bailey 1979, Heitmeyer 1985, Austin 1988) and likely serves as insulation during fall and winter.

Detecting seasonal differences in fatty acid composition of waterfowl can be confounded by slow turnover rates in adipose tissue, degree of change in dietary fatty acids, and the investigator's sampling schedule relative to changes in a bird's diet. However, the lack of seasonal or sexual differences in fatty acid composition of geese in this study was expected because of their diverse diets (Eggeman et al. 1989) and habitat use (Austin 1988). Fatty acid composition of other avian species also becomes more uniform when diets are diversified, such as in Common Redpolls (*Carduelis flammea*) (West and Meng 1968) and Northern Bobwhite (*Colinus virginianus*) (Robel and Klopstein 1985).

Of the five fatty acids that varied among years, C18:3, a typical chloroplast fatty acid (Tinoco 1982), was unique in that its level was highest

in 1984–1985. This reflects the greater availability and frequency of use of winter wheat forage by Canada Geese in that year (Austin 1988).

In contrast, C14:0, C16:0, C16:1, and C18:1 were highest in 1986–1987 when cornfields received less use and other habitats, such as moist-soil units, received greater use (Austin 1988). C14:0 occurs at relatively high levels in soybeans (Cherry et al. 1985) and some moist-soil foods (Heitmeyer and Fredrickson 1990) but does not occur in corn (National Research Council 1984). Canada Geese and other waterfowl can use native, moist-soil foods extensively (Fredrickson and Taylor 1982, Austin et al. 1993), but few of these foods have been evaluated for their fatty acid composition and nutritional value (Matthiesen and Stoller 1978, Heitmeyer and Fredrickson 1990).

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